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**14. ABSTRACT**

For the first time reductive dechlorination of PCBs was shown to occur in marine and estuarine microcosms from sites with ambient temperatures below 10 °C. It was shown that the types of activities are influenced by the location of the site and the ambient temperature. In addition, it is now clear that non-indigenous dechlorinating organisms can be added to sediments microcosms to dechlorinate PCBs. These studies suggest that reductive dechlorination of PCBs has the potential to occur in coastal regions with lower ambient temperatures. The result also suggest that active biotreatment of PCB impacted dredge spoils by augmentation may be possible in colder climates.

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**FINAL REPORT**

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**GRANT TITLE:** Collaborative Research: Reductive Dechlorination of Polychlorinated Biphenyls (PCBs) in Marine Harbor Sediments

**REPORT PERIOD:** 01 June 1998 - 31 May 2002

**OBJECTIVE:** The overall goal of the project was to determine the intrinsic dechlorinating activities in low temperature marine sediments from Norway and identify the types of microorganisms that catalyze these reactions.

**APPROACH:** Enrichment culture techniques were combined with molecular monitoring using 16S rDNA gene probes to develop highly defined PCB dechlorinating consortia of microorganisms from Norwegian sediments. Highly enriched microcosms were developed at various temperatures (i.e., 4, 10, and 20°C) in minimal medium inoculated from several PCB impacted coastal sites. The low temperature Norwegian sites in Trondheim, Bergen and Oslo harbors were impacted by PCBs by a range of activities that included WWII submarine bunkers, gas plant, heavy industry and shipping. Concurrently, enrichment microcosms from Baltimore Harbor were developed to determine whether low temperature dechlorination was intrinsic to low temperature sites only and to compare the microbial communities from psychrophilic and mesophilic sediments.

This approach was coordinated through the complementary efforts of an international collaboration between SINTEF in Oslo, Norway, and the Medical University of South Carolina (MUSC) and University of Maryland Biotechnology Institute (UMBI) in the USA. Dr. Briseid of SINTEF was responsible for the collection and analytical characterization of Norwegian sediments impacted by PCBs. Dr. May of MUSC developed the enrichment microcosms with PCB impacted sediments and characterized the dechlorination reactions. Dr. Sowers conducted molecular analysis of the microbial communities in the PCB dechlorinating microcosms and coordinated the efforts of the collaborators.

**ACCOMPLISHMENTS:**

***Intrinsic PCB Dechlorination in Low Temperature Sediments.*** In the first year of ONR funded research, the PIs have developed enrichment cultures with single congeners and sediment samples from Oslofjorden in Norway. The cultures exhibited intrinsic activity on congener 2,3,4,5-CB. The dechlorination is focused on the *para* position on the biphenyl ring and no *ortho* dechlorination was observed. This activity was independently confirmed by collaborators in Norway and the US. This was the first intrinsic dechlorination observed with Norwegian coastal sediments and the results showed that intrinsic bioremediation of a site may occur. Although intrinsic PCB dechlorination was observed in sediments from the selected sites, only limited activity, dechlorination of the *para* chlorine, was observed.

Having established dechlorinating activity in Norwegian sediments from this initial test site, collaborators from the US (K. Sowers and H. May) visited Norway from August 28 - September 6, 1999 to meet with collaborators and to collect sediment samples from several locations along

the Western coast of Norway to determine the extent of PCB contamination and range of dechlorinating activities in PCB impacted sites. SINTEF collaborators arranged sampling cruises in Trondheim and Bergen. These sediments, along with those previously collected in Oslo Fjord, were screened for their PCB-dehalogenating potential in accordance with Objective 1 of the original NICOP proposal.

Sediments were collected from 4 sites: Nyhavna (N) and Hurtigrutekaia (H) in Trondheim Fjord; Laksevåg (L) and Solheimsviken (S) near Bergen Harbor. Each sediment was incubated in triplicate in E-Cl medium under anaerobic conditions with 2.5 mM each of sodium acetate, butyrate and propionate, plus 50 µg/ml of 2,3,4-CB, 2,3,4,5-CB and 2,3,4,5,6-CB. In order to fulfill Objective 3 (identification of factors that influence dechlorination at these sites), sediment microcosms were incubated at different temperatures (4, 10, 25 and 30°C). The cultures were incubated for up to 6 months while methane and PCBs were analyzed. Sediments from Baltimore Harbor were examined in parallel for comparison with the Norwegian sediments. Transfers were made from the 4 and 10°C cultures at 6 months. Some of the results of these experiments are presented below.

Active dechlorination of 2,3,4,5-CB was not observed initially with any of the sediments at 4°C. However, traces of product were seen at 182 days in a few of the microcosms. In contrast, sediment from 3 out of the 4 sites showed significant dechlorinating activity at 10 °C, which was near the ambient temperature (10 - 12°C) at each site. Sediment form the 4$^{th}$ site, H sediment, showed some activity in 2 out of 3 tubes. Interestingly, more dechlorination was observed with B sediment at 10°C than any other sediment, thus opening up the possibility of using organisms from this site to augment a colder temperature site such as in Norway (see Bioaugmentation results below).

Baltimore Harbor (B) sediment is known to harbor *ortho* PCB dechlorinating bacteria. But we observed that *ortho* dechlorination with B sediment is favored at 25°C versus 30°C and that it was completely absent at 10°C (*meta* and *para* dechlorination did occur at this temperature). PCB dechlorination was also expressed in microcosms with N and H sediments. The acclimation time preceding dechlorination was significantly longer than that observed with B sediment. However, overall dechlorination with N sediments was nearly equal to that of B. More importantly, a wider variety of dechlorination products were observed with N and H sediments at this temperature, including some *ortho* dechlorination products. The late and occasional observation of *ortho* dechlorination at 10°C in N and H sediments may mean that *ortho* dechlorinating bacteria different from strain *o*-17 exist in these sediments. Interestingly, no dechlorination was observed with S sediments at 4 and 10°C. Yet, these sediments did express dechlorination at 25 and 30°C. As expected, B sediment showed strong dechlorination at the higher temperatures, although *ortho* dechlorination declined somewhat at 30°C. In contrast, dechlorination with L, H and N sediments generally declined at the higher temperatures. In fact, L sediment only showed a trace of dechlorination at 25°C and nothing at 30°C. Although the activity at 10°C was not robust with L sediments, it was significantly more than what was observed at the higher temperatures. N sediment, which expressed relatively strong and varied dechlorination at 10°C lost much of this capability at 25 and 30°C. Therefore, the assumption that an increase in temperature will enhance PCB dechlorination should be carefully examined before proceeding with a remediation attempt.

To test for reproducibility PCB dechlorinating microcosms were sequentially transferred 3 additional times. There was dechlorination at 10°C with both Baltimore and Nyhavna enrichment cultures after two transfers. This is consistent with the initial enrichment cultures. In general the types of activity are the same but some important changes have occurred. The Baltimore cultures at 25°C did show the most dechlorination, but the activity was slower than that of the initial set

of enrichment cultures and slower than what we routinely see with such an enrichment culture. In addition, the *ortho* dechlorination had declined significantly. We did analyze these cultures much later (425 days) and they showed more *ortho* dechlorination and more overall dechlorination. The onset and rate of dechlorination with Nyhavna at 25°C was about the same as it was in the initial enrichment cultures. However, the initial cultures only exhibited *para* dechlorination. The 3rd set of cultures showed *para* and *meta* dechlorination, *meta* favored.

Baltimore showed the most dechlorination at 10°C in initial cultures. The results with the 3rd set of these cultures were somewhat mixed. Some cultures were not very active while others dechlorinated more rapidly (or at least with a shorter lag) than the initial enrichment cultures. The types of dechlorination observed remained the same after the two transfers (3rd set). When compared with other Norwegian sediments the Nyhavna enrichment cultures showed the best dechlorination at 10°C in initial cultures. The 3rd set of these enrichment cultures had a shorter lag (75 days or less) compared with the initial cultures (>110 days). This suggests that cold psychrophilic or psychrotrophic organisms could be enriched and that they may adapt. It would be interesting to determine if this dechlorination could continue to be enhanced and to determine what population would arise. However, in addition to the shorter lag time, the 3rd set of Nyhavna cultures lost the ability to *ortho* dechlorinate.

In the earlier enrichment microcosms we only observed traces of products with enrichment cultures incubated at 4°C. The dechlorination patterns were the same as those observed at 10°C, but occurred at a lower rate. However, significantly greater dechlorination occurred in 4°C cultures that were transferred from microcosms incubated at 10°C. If this observation holds, then it could be that by first adapting the cultures to 10°C before incubation at 4°C it is possible to enhance low temperature dechlorination in microcosms.

The effect of sulfate was also tested on microcosms dechlorinating both single congeners (C21, C54, C116, C169, C186) and Aroclor 1242. The purpose was to determine whether dechlorination rates and pathways are affected differently by concentrations that are characteristic of marine, estuarine and freshwater environments typically found in harbor regions. Results of these studies showed that higher concentrations of sulfate delayed the dechlorination of Aroclor 1242 and individual congeners in microcosms. Since dechlorination was delayed, but not completely inhibited the results suggest that electron donors become limiting in sulfate-rich environments.

***Molecular Analysis.*** Molecular probes were used to analyze the 16S rDNA of microbial populations in the low temperature PCB dechalogenating cultures. Universal primers were used to assess the overall diversity of the microbial populations. In these studies the presence of 2345-CB caused only a slight shift in the microbial populations indicating that the congener was not toxic to the general population. The negligible shift detected in the microbial population in response to the PCB congener is consistent with previous reports by the PIs indicating that PCB dechlorinating species do not predominate the population during active dechlorination in initial microcosms. The microbial population was significantly affected by temperature. In studies with both Nyhavna and Baltimore Harbor microcosms incubated at 10 and 25°C the microbial diversity was reduced at lower temperatures. A third observation in population assessment studies was that there was generally a difference in the microbial communities between Nyhavna and Baltimore Harbor.

Microcosms were also probed with 16S rDNA probes that were selective for PCB dechlorinating microorganisms (o-17 and DF-1) and closely related species (*Dehalococcoides*) that dehalogenate other chlorinated compounds. Surprisingly a signal indicating the presence of either group of microorganisms was not detected in any actively dechlorination microcosms with Nyhavna sediment, but PCR products were detected with universal primers (data not shown). Factors that may explain this observation are: 1) inhibitory compounds in the reaction mixture prevented PCR

amplification with the specific primers by reducing the hybridization efficiency, but allowed priming by the universal primers; 2) the dehalogenating microorganisms are too dissimilar from known PCB dechlorinators to hybridize with the selective primers. We are currently testing additional hybridization conditions to promote hybridization including sample dilution, purification with molecular exclusion resin and modified primers. As we discover additional PCB dechlorinating microorganisms in related studies and obtain more sequence data to make modifications to the consensus primer sequences, we will test these modified probes with the Nyhavna microcosms.

***Bioaugmentation.*** During our examination of Norwegian Harbor sediments we have discovered that *ortho* dechlorination is rare to non-existent. In preliminary experiments the PIs successfully introduced the *ortho* dechlorinating culture from Baltimore Harbor into Oslo Harbor sediments. The reductive dechlorination of the PCB congener was monitored over the course of 3 months. These experiments established that bioaugmentation with highly enriched PCB-dechlorinating sediment could be used to introduce PCB-dechlorination into non-intrinsic sediments.

In order to determine whether PCB dechlorinating microorganisms were stable with intrinsic microbial populations the initial microcosms were transferred to fresh medium and incubated in the absence of PCB. After incubation for a period of 120 days fatty acids and 2356-CB were added to the microcosms and dechlorinating activity was monitored for an additional 120 days. No dechlorinating activity was detected and bacterium o-17 was not detected by DGGE analysis, which suggests that the PCB dechlorinating microorganisms introduced into the sediments did not survive in the absence of PCB.

**CONCLUSIONS:** For the first time reductive dechlorination of PCBs was shown to occur in marine and estuarine microcosms from sites with ambient temperatures below 10 °C. It was shown that the types of activities are influenced by the location of the site and the ambient temperature. In addition, it is now clear that non-indigenous dechlorinating organisms can be added to sediments microcosms to dechlorinate PCBs. These studies suggest that reductive dechlorination of PCBs has the potential to occur in coastal regions with lower ambient temperatures. The result also suggest that active biotreatment of PCB impacted dredge spoils by augmentation may be possible in colder climates.

**SIGNIFICANCE:** Results indicating that low temperature dechlorinations of PCBs occurs and exhibits dechlorinating patterns different from those observed in sediments from temperate climates indicates that there is the potential for natural attenuation of PCBs in cooler climates, such as those found in northern Europe, America and the Arctic.

**PATENT INFORMATION:** Information from this project contributed to a patent that relates to the use of microbes to catalyze dechlorination of PCBs.

**AWARD INFORMATION:** The PI (KRS) and the co-PI (HDM) were promoted to the rank of associated professor during the course of this project.

**PUBLICATIONS:**

Fagervold, S., T. Briseid, O. Bergersen, and G. Eidsa. 2000. Dechlorination of Aroclor and specific congeners in marine, estuarine and freshwater sediments. Proc. 2nd Int. Conf. on Remediation of Chlorinated and Recalcitrant Compounds. Monterey, CA May 22-25. Eds. Wickramanayake, G.B. and Gavaskar, A.R., ISBN: 1-57477-094.

May, H.D., T. Briseid, O. Bergersen, G. Eidsa and K.R. Sowers. Effects of temperature on the reductive dechlorination of PCBs in microcosms from low temperature marine sediments. *In preparation.*

Watts, J.E.M., H.D. May, T. Briseid, G. Eidsa and K.R. Sowers. Distribution of PCB dechlorinating microbes in Norwegian coastal sediments. *In preparation.*